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COUNSELORS AT LAW

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Scott C. Bovino
215-963-5071

October 15, 1997

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Document Control Office
Attention: Section 8(e) Coordinator
Office of Pollution Prevention and Toxics (7407)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Re: TSCA Section 8(e) Notice for CAS Registry Number Substance 172491-73-5

EPA Section 8(e) Coordinator:

Enclosed is a bacterial mutagenicity assay report prepared by Huntingdon Life Sciences Ltd. for TDK Corporation of Japan ("TDK Japan"). This mutagenicity study indicates that CAS Registry Number Substance (referred to as in the study; hereinafter referred to as the TDK Substance), when tested in dimethyl formamide, shows evidence of mutagenic activity. TDK Electronics Corporation ("TEC") received a copy of the mutagenicity study from TDK Japan on July 29, 1996. TEC submitted a low volume exemption ("LVE") notice for the TDK Substance on April 3, 1996. That LVE notice cleared EPA review on May 3, 1996.

TEC initially evaluated the mutagenicity study for the TDK Substance in accordance with EPA's June 1991 TSCA Section 8(e) Reporting Guide (the "1991 Section 8(e) Guidance"). With respect to mutagenicity studies, the 1991 Section 8(e) Guidance (pg. 23) states, in relevant part, as follows:

> "[A] positive in vitro genotoxicity test, when considered alone, is usually insufficient to cause reporting under Section 8(e). However, EPA believes that such information is of value in assessing the possible

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> risk(s) posed by exposure to the tested chemical or mixture. Further, the Agency believes that a positive in vitro genotoxicity test result, in combination with other information (e.g., knowledge of actual/potential exposure to and/or high production of the tested chemical), would suggest the need, in many cases, to conduct further studies designed to determine the toxicity of or the exposure to that chemical.

Language identical to that cited above is also contained in a July 1989 TSCA Section 8(e) Question & Answer document issued by EPA (the "1989 Section 8(e) Q&A").

Based on the 1991 Section 8(e) Guidance, TEC concluded that it was not required to report the mutagenicity study for the TDK Substance under Section 8(e) of TSCA. In reaching this conclusion, TEC considered that: (1) the TDK Substance is imported in to the United States in small quantities (the LVE notice limit is); and (2) actual/potential exposure to the TDK Substance is extremely low (the LVE notice indicates that two workers, wearing protective equipment, are exposed to the substance for a maximum of hours per day).

Recently, however, TEC obtained from EPA's TSCA Hotline a copy of a document entitled "EPA Response to December 26, 1991 'Working Paper'" (the "Section 8(e) Working Paper Response"). This document contains the following language concerning the reporting of mutagenicity studies under Section 8(e) of TSCA:

> EPA's position has been and continues to be that a single positive in vitro genotoxicologic test finding, when considered alone, is typically not sufficient to offer reasonable support for a conclusion of substantial risk as those terms are used and described in the Agency's March 16, 1978, Section 8(e) policy statement . . . . It should be noted, however, that there are certain circumstances (e.g., a positive in vitro result that exceeds the positive control or a strong positive in vitro result involving a chemical to which there is actual wide-spread human exposure) that should receive immediate consideration for reporting under TSCA Section 8(e). Further, the Agency believes that a positive in vitro test result (e.g., an Ames mutagenicity test), when combined with knowledge of 1) actual or potential exposure to the subject chemical, or 2) high production levels of the subject chemical, indicates the need to conduct further genotoxicity and/or other toxicologic testing.

The Section 8(e) Working Paper Response appears to establish an additional situation for which reporting of a mutagenicity study is required under Section 8(e) of TSCA. Specifically,

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the Section 8(e) Working Paper Response indicates that reporting may be required if the study demonstrates "a positive in vitro result that exceeds the positive control." Reporting in such a situation is clearly not contemplated under the 1991 Section 8(e) Guidance or the 1989 Section 8(e) Q&A. If EPA intends mutagenicity studies demonstrating "a positive in vitro result that exceeds the positive control" to be reportable under Section 8(e) of TSCA, a substantially more definitive pronouncement of this policy is required.

In addition, to the extent a mutagenicity study with "a positive in vitro result that exceeds the positive control" is reportable under Section 8(e) of TSCA, TEC is unable to determine whether the mutagenicity study for the TDK Substance is reportable. The Section 8(e) Working Paper Response does not explain when a positive in vitro result actually "exceeds" the positive control. A positive control is tested at a single, low concentration to determine whether the bacterial system being utilized is active. In contrast, a mutagenicity test substance is analyzed at various concentrations to determine whether an increase in concentration correlates with an increase in gene mutations. Typically, the concentrations at which the test substance is analyzed are much higher than the concentration at which the positive control is tested. Because of this difference in concentration, it makes no sense to ask whether the positive in vitro result "exceeds" the positive control. There is simply no reasonable basis by which to compare the test results for two substances analyzed at substantially different concentrations.

The mutagenicity study for the TDK Substance illustrates this point. The number of reverse mutation colonies per plate detected for the TDK Substance was greater than the number of reverse mutation colonies per plate detected for the positive control in two isolated situations (see pg. 21--TA 100 and pg. 25--TA 98). Neither of these test results was duplicated when the mutagenicity test was repeated. Furthermore, both test results were generated when the TDK Substance was tested at 5000 µg/plate, its highest concentration. In contrast, the positive control was tested at concentrations as low as 3.0 µg/plate and 0.5 µg/plate. Clearly, it is meaningless to compare such test figures.

In sum, TEC does not know whether EPA would consider the results of the mutagenicity study for the TDK Substance to be indicative of "a positive in vitro result that exceeds the positive control." Nevertheless, in an abundance of caution, TEC is submitting a copy of the mutagenicity study to EPA under Section 8(e) of TSCA. After reviewing this information, we hope that EPA will issue guidance clarifying when a positive in vitro result "exceeds" the positive control. In addition, we think it would be helpful if EPA publicized more widely and definitively its statement that such mutagenicity studies are, in fact, reportable under Section 8(e) of TSCA.

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Please note that copies of this letter and the mutagenicity study with all confidential business information deleted are included for filing in the public docket. If you have any questions or comments, please feel free to contact me. Thank you for your attention to this matter.

Sincerely,

Scott C. Bovino

NOTE

This report is considered by the Study Director to be the 'final draft' and has been submitted to the Huntingdon Life Sciences Quality Assurance Department for Audit.

The sponsor is requested to review this document and communicate any comments to the Study Director as soon as possible. When these comments have been received and on completion of the QA audit, the FINAL REPORT containing Study Director and QA Statements will be issued.

PLEASE NOTE

In compliance with GLP any changes to the final report after the date of issue will be in the form of a separate amendment to the report.

Date: 15 July 1996 V.1.

BACTERIAL MUTATION ASSAY

Sponsor

1st Technical Center,
TDK Corporation,
462-1 Otai,
Saku,
Nagano-ken 389-02,
JAPAN.

Testing facility

Huntingdon Life Sciences Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued

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TABLES

APPENDIX

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register, 22 December 1978, and subsequent amendments.

Japan Ministry of Health and Welfare, Notification No. Yakuhatsu 313 Pharmaceutical Affairs Bureau, 31 March 1982 and subsequent amendment Notification No. Yakuhatsu 870, Pharmaceutical Affairs Bureau, 5 October 1988.

.....

Ricarda A. Gant, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

Date

QUALITY ASSURANCE STATEMENT

This report has been audited by Huntingdon Life Sciences Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Certain studies such as that described in this report, are conducted at Huntingdon in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to Management, Huntingdon Life Sciences.

Date(s) of inspection

Date(s) of reporting inspection findings to the Study Director and Management

Date of reporting audit findings to the Study Director and Management

.....

Rod Scammell,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

Date

RESPONSIBLE PERSONNEL

Ricarda A. Gant, B.Sc. (Hons.),
Study Director,
Department of Cellular Toxicology. .....

SUMMARY

In this *in vitro* assessment of the mutagenic potential of , histidine dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA 1535, TA 1537, TA 98 and TA 100) and a tryptophan dependent mutant of *Escherichia coli* (WP2 *uvr*A) were exposed to the test substance, diluted in dimethyl formamide which was also used as a negative control.

Two independent mutation tests were performed, in the presence and absence of liver preparations from Aroclor 1254-induced rats.

In the preliminary toxicity test with dose levels of up to 5000 µg active ingredient/plate no toxicity was observed. A top dose level of 5000 µg active ingredient/plate was chosen for the subsequent mutation study. Other dose levels used in the mutation assays were: 2500, 1250, 625 and 312.5 µg active ingredient/plate.

Substantial dose-related increases in revertant colony numbers were observed with TA 1535, TA 98 and TA 100 in the presence of S-9 mix.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

It is concluded that, when tested in dimethyl formamide, shows evidence of mutagenic activity in this bacterial system.

INTRODUCTION

In the *in vitro* technique described by Ames and his co-workers, (Ames, McCann and Yamasaki 1975, Maron and Ames 1983) mutagenic effects are determined by exposing mutant strains of *Salmonella typhimurium* to various concentrations of the test substance. Normally *S. typhimurium* is capable of synthesising the amino acid histidine it requires for growth but the mutant strains used in this test are incapable of this function. When large populations of these strains are exposed to a mutagen, reverse mutation to the original histidine independent form takes place in a proportion of the bacterial population. These colonies (revertant) are readily detectable due to their ability to grow on a histidine deficient medium.

A technique based on similar principles has also been described by Green (1984). This system employs mutant strains of *Escherichia coli* which are incapable of synthesising the amino acid tryptophan required for growth.

The strains of *S. typhimurium* routinely used carry additional mutations which render them more sensitive to mutagens. All strains have a defective cell coat which allows greater permeability of test substances into the cell. All strains are deficient in normal DNA repair processes. In addition, strains TA 98 and TA 100 possess a plasmid (pKM101) which introduces an error-prone repair process, resulting in increased sensitivity to mutagens. The strain of *E. coli* used is defective in DNA repair processes.

Since many substances do not exert their mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell, the test substance and the bacteria are incubated in the presence of a supplemented liver fraction (S-9 mix) prepared from rats previously treated with a compound (Aroclor 1254) known to induce a high level of enzymic activity.

This report describes a study designed to comply with the following guidelines:

OECD Guidelines for Testing of Chemicals No. 471: Genetic Toxicology: *Salmonella typhimurium*, Reverse Mutation Assay, 26 May 1983,

OECD Guidelines for Testing of Chemicals No. 472: Genetic Toxicology: *Escherichia coli*, Reverse Mutation Assay, 26 May 1983,

EEC Methods for Determination of Toxicity, Annex to Directive 92/69/EEC, (OJ No. L383A, 29.12.92), Part B, Method B.14. Other effects - Mutagenicity: *Salmonella typhimurium* - Reverse Mutation Assay,

EEC Methods for Determination of Toxicity, Annex to Directive 92/69/EEC, (OJ No. L383A, 29.12.92), Part B, Method B.13. Other effects - Mutagenicity: *Escherichia coli* - Reverse Mutation Assay,

US Environmental Protection Agency, Method: HG-Gene Muta - *S. typhimurium*: The *Salmonella typhimurium* reverse mutation assay, 1984,

Japanese, Ministry of Agricultural, Forestry and Fisheries: Notification of Director General, Agricultural Production Bureau, NohSan 4200, 28 January 1985,

Japanese, Ministry of Health and Welfare, Notification Yakushin 1 No. 24, 11 September 1989 Guidelines for Toxicity Studies of Drugs, 4 I, Bacterial Reverse Mutation Test,

Japanese, Ministry of International Trade & Industry, 61 Kikyoku No. 1014, 5 December 1986 and 62 Kikyoku No. 303, 31 March 1987,

Japanese, Ministry of Labour, Guidebook of Mutagenicity Tests, published 16 June 1987.

The protocol was approved by the Study Director on 28 May 1996, by Huntingdon Life Sciences Management on 21 December 1995 and by the Sponsor on 24 May 1996.

The experimental phase of the study was conducted between 4 and 28 June 1996.

TEST SUBSTANCE

Identity:	
Chemical name:	
Intended use:	Information not available to Huntingdon Life Sciences
Appearance:	powder
Storage conditions:	Room temperature in the dark
Batch number:	/G1113A1870
Expiry date:	Twelve months from date of receipt
Purity:	89.0%
Date received:	11 December 1995
Supplier:	TDK Corporation

EXPERIMENTAL PROCEDURE

BACTERIAL STRAINS

The following strains of *S. typhimurium* were used in the test:

S. typhimurium TA 1535 *hisG46 rfa uvr*B
S. typhimurium TA 1537 *hisC3076 rfa uvr*B
S. typhimurium TA 98 *hisD3052 rfa uvr*B pKM101
S. typhimurium TA 100 *hisG46 rfa uvr*B pKM101

The strains were obtained from Professor B.N. Ames, University of California, California, USA.

The strain of *E. coli* used was:

E. coli WP2 *uvr*A *trp*

It was obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland.

Batches of the strains were stored at −80°C. Each batch of frozen strain was tested for cell membrane permeability and, where applicable, for the pKM101 plasmid which confers resistance to ampicillin. The response of the strains to a series of diagnostic mutagens was also assessed.

For use in tests an aliquot of frozen culture was added to 25 ml of nutrient broth (DAB 7, Merck) and incubated, with shaking, at 37°C for 10 hours. These cultures provided at least 2×10^9 cells per ml which were measured photometrically.

POSITIVE CONTROL COMPOUNDS

In the absence of S-9 mix

Identity	*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine
Supplier	Sigma
Batch	67F-3700
Appearance	Pale yellow crystalline powder
Solvent	Dimethyl sulphoxide
Concentration	5 µg/plate for strain TA 1535 3 µg/plate for strain TA 100 2 µg/plate for strain WP2 *uvr*A

Identity	9-Aminoacridine
Supplier	Sigma
Batch	96F-05641
Appearance	Yellow powder
Solvent	Dimethyl sulphoxide
Concentration	80 μg/plate for strain TA 1537

Identity	2-Nitrofluorene
Supplier	Aldrich
Batch	61896
Appearance	Beige powder
Solvent	Dimethyl sulphoxide
Concentration	1 μg/plate for strain TA 98

In the presence of S-9 mix

Identity	2-Aminoanthracene
Supplier	Aldrich
Batch	0013406
Appearance	Green powder
Solvent	Dimethyl sulphoxide
Concentration	2 μg/plate for strains TA 1535 and TA 1537 0.5 μg/plate for strain TA 98 1 μg/plate for strain TA 100 10 μg/plate for strain WP2 *uvr*A

PREPARATION OF S-9 FRACTION

Species	Rat
Sex	Male
Strain	Sprague-Dawley derived
Source	Harlan Olac Ltd
Age	7-8 weeks
Weight	<300 g
Diet	Biosure Rodent Diet LAD 1

Mixed function oxidase systems in the livers of a group of rats were stimulated by Aroclor 1254, administered as a single intra-peritoneal injection in Arachis oil at a dosage of 500 mg/kg bodyweight. On the fifth day after injection, following an overnight starvation, the rats were killed, and their livers aseptically removed.

The following steps were carried out at 0-4°C under aseptic conditions. The livers were placed in 0.15 M KCl (3 ml KCl : 1 g liver) before being transferred to an Ultra-Turrax homogeniser. Following preparation, the homogenates were centrifuged at 9000 *g* for 10 minutes. The supernatant fraction (S-9 fraction) was dispensed into aliquots and stored at −80°C until required. The efficacy of each batch of S-9 fraction was tested with the carcinogens 7,12-dimethylbenzanthracene and 2-aminoanthracene before use.

PREPARATION OF S-9 MIX

S-9 mix contained: S-9 fraction (10% v/v), $MgCl_2$ (8 mM), KCl (33 mM), sodium phosphate buffer pH 7.4 (100 mM), glucose-6-phosphate (5 mM), NADPH (4 mM), NADH (4 mM). All the cofactors were filter-sterilised before use.

SELECTION OF SOLVENT

Prior to commencing testing the solubility of the test substance was assessed. At 56.18 mg/ml (equivalent to 50 mg active ingredient/ml) was insoluble in dimethyl sulphoxide, ethanol and acetone, and soluble in dimethyl formamide. Solubility was not assessed in water as the Sponsor had indicated that was not soluble in aqueous solvents. Therefore dimethyl formamide was the chosen solvent for use in subsequent tests.

PRELIMINARY TOXICITY TEST

Four concentrations of test substance were assessed for toxicity using the five tester strains. The highest concentration was 50 mg active ingredient/ml of test substance in the chosen solvent, which provided a final concentration of 5000 µg active ingredient/plate. Three 10-fold serial dilutions of the highest concentration were also tested. The chosen solvent, dimethyl formamide, was used as the negative control.

An aliquot of 0.1 ml of a 10 hour bacterial culture and 0.5 ml S-9 mix or 0.5 ml 0.1 M phosphate buffer (pH 7.4) were placed in glass bottles. An aliquot of 0.1 ml of the test solution was added, followed immediately by 2 ml of histidine/tryptophan deficient agar. The mixture was thoroughly shaken and overlaid onto previously prepared petri dishes containing 25 ml minimal agar. A single petri dish was used for each dose level. Plates were also prepared without the addition of bacteria in order to assess the sterility of the test substance, S-9 mix and phosphate buffer. All plates were incubated at 37°C for 3 days. After this period the appearance of the background bacterial lawn was examined. Revertant colonies were counted using a Seescan Automatic Colony Counter.

Any toxic effects of the test substance can be detected by a substantial reduction in revertant colony counts or by the absence of a complete background bacterial lawn. In the absence of any toxic effects the top concentration used in the main tests is the same as that used in the preliminary toxicity test. If toxic effects are observed a lower concentration may be chosen for the main assays. Ideally the concentrations chosen for the mutation tests should include a minimum of four non-toxic concentrations.

MUTATION TEST PROCEDURE

The test substance was added to cultures of the five tester strains at five concentrations separated by 2-fold dilutions. The highest concentration of used was 5000 μg active ingredient/plate. The negative control was the chosen solvent, dimethyl formamide. The positive control compounds were also included.

An aliquot of 0.1 ml of a 10 hour bacterial culture and 0.5 ml S-9 mix or 0.5 ml 0.1 M phosphate buffer (pH 7.4) were placed in glass bottles. An aliquot of 0.1 ml of the test solution was added, followed immediately by 2 ml of histidine/tryptophan deficient agar. The mixture was thoroughly shaken and overlaid onto previously prepared petri dishes containing 25 ml minimal agar. Three petri dishes were used for each dose level. A set of plates were also prepared containing only bacterial culture and S-9 mix or phosphate buffer (0 μg/plate). Plates were also prepared without the addition of bacteria in order to assess the sterility of the test substance, S-9 mix and phosphate buffer. All plates were incubated at 37°C for 3 days. After this period revertant colonies were counted using a Seescan Automatic Colony Counter.

At a later date the main test was repeated using the procedures described above with the same concentrations of test substance.

STABILITY AND FORMULATION ANALYSIS

The stability of the test substance and of the test substance in the solvent were not determined as part of this study. Analysis of achieved concentration was not performed as part of this study.

ASSESSMENT OF RESULTS

The mean number of revertant colonies for all treatment groups is compared with those obtained for solvent control groups. The mutagenic activity of a test substance is assessed by applying the following criteria:

(a) If treatment with a test substance produces an increase in revertant colony numbers of at least twice the concurrent solvent controls, with some evidence of a positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S-9 mix, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(b) If treatment with a test substance does not produce reproducible increases of at least 1.5 times the concurrent solvent controls, at any dose level with any bacterial strain, it is considered to show no evidence of mutagenic activity in this test system. No statistical analysis is performed.

(c) If the results obtained fail to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), the following approach is taken in order to resolve the issue of the substance's mutagenic activity in this test system.

(i) Repeat tests may be performed using modifications of the experimental method. These modifications include (but are not restricted to), the use of a narrower dose range than that already tested; the use of different levels of liver homogenate S-9 fraction in the S-9 mix. Should an increase in revertant colony numbers be observed which satisfies paragraph (a) the substance is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(ii) If no clear "positive" response can be obtained the test data may be subjected to analysis to determine the statistical significance of any observed increases in revertant colony numbers. The statistical procedures used will be those described by Mahon *et al.* (1989) and will usually be analysis of variance followed by Dunnett's test.

ARCHIVES

All data are kept in a loose-leaved laboratory notebook which is held in the Department of Cellular Toxicology and later transferred, together with a copy of the final report, to the Archive Department, Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, UK for a minimum period of five years. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's knowledge.

RESULTS

The revertant colony counts for obtained in the preliminary toxicity test are shown in Table 1. was not toxic towards the tester strains. Therefore 5000 µg active ingredient/plate was chosen as the top dose level in the mutation tests.

The mean numbers of revertant colonies obtained in the first mutation test are shown in Table 2.

The mean number of revertant colonies, together with the individual plate counts for obtained in the first mutation test with the tester strains are shown in Table 3. Positive control mutability checks are shown in Table 4.

Following treatment with in the first main mutation test, substantial dose-related increases in revertant colony numbers were observed with TA 100 and TA 1535 in the presence of S-9 mix.

The mean numbers of revertant colonies obtained in the second mutation test are shown in Table 5.

The mean number of revertant colonies, together with the individual plate counts for obtained in the second mutation test with the tester strains are shown in Table 6. Positive control mutability checks are shown in Table 7.

Following treatment with in the second mutation test, substantial dose-related increases in revertant colony numbers were observed with TA 100, TA 98 and TA 1535 in the presence of S-9 mix. A single increase in colony numbers was also seen with TA 100 at 5000 µg active ingredient/plate.

The concurrent positive control compounds demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

CONCLUSION

It is concluded that, when tested in dimethyl formamide, shows evidence of mutagenic activity in this bacterial system.

REFERENCES

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EEC (1993) EEC Methods for Determination of Toxicity, Annex to Directive 92/69/EEC, (OJ No. L383A, 29.12.92), Part B, Method B.13. Other effects - Mutagenicity: *Escherichia coli* - Reverse Mutation Assay.

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MAHON, G.A.T., GREEN, M.H.L., MIDDLETON, B., MITCHELL, I.DE G., ROBINSON, W.D. and TWEATS, D.J. (1989) Analysis of data from microbial colony assay in: KIRKLAND, D.J. (Ed.) *UKEMS Subcommittee on Guidelines for Mutagenicity Testing. Report, Part III. Statistical Evaluation of Mutagenicity Test Data*, p.26. Cambridge University Press, Cambridge.

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OECD (1983) OECD Guidelines for Testing of Chemicals No. 472: Genetic Toxicology: *Escherichia coli*, Reverse Mutation Assay, 26 May 1983.

FIGURE 1

Mutation Test 1 -S-9 mix

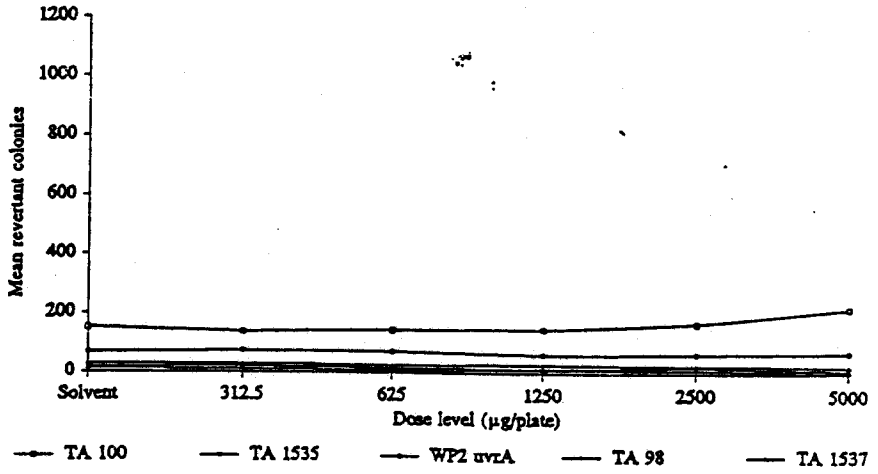

FIGURE 2

Mutation Test 1 +S-9 mix

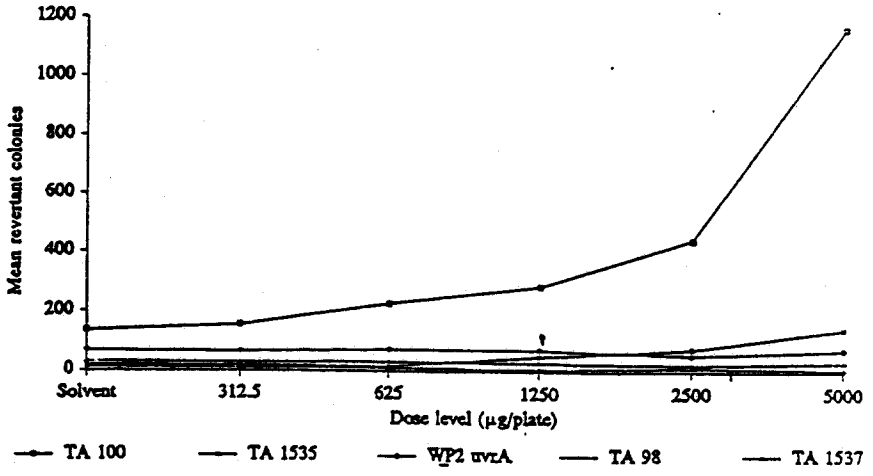

FIGURE 3

Mutation Test 2 -S-9 mix

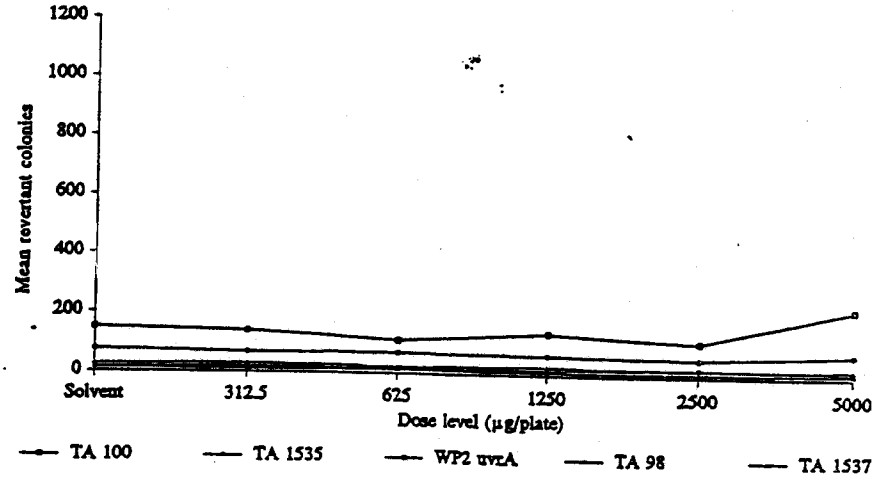

FIGURE 4

Mutation Test 2 +S-9 mix

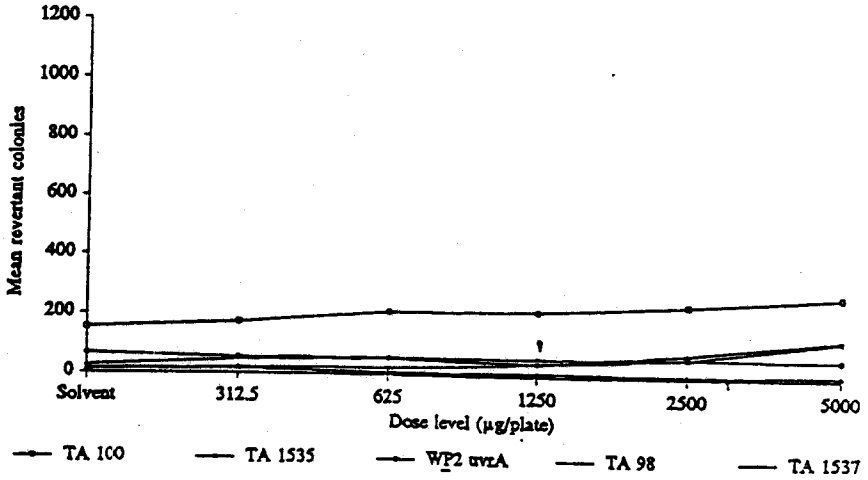

TABLE 1

Preliminary toxicity test on
Revertant colony counts obtained

Material	Test concentration (μg active ingredient /plate)	With or without S-9	Reverse mutation (number of colonies/plate) *				
			Base pair exchange type			Frame shift type	
			TA 100	TA 1535	WP2 uvrA	TA 98	TA 1537
Solvent control		-	121	14	57	24	15
	5000	-	97P	15P	83P	31P	13P
	500	-	168P	23P	58P	29P	13P
	50	-	132	14	57	31	8
	5	-	132	23	52	31	5
Solvent control		+	116	20	54	22	17
	5000	+	1150P	108P	69P	37P	19P
	500	+	201P	18P	53P	34P	11P
	50	+	144	20	54	30	5
	5	+	133	16	58	23	7

− Absence
+ Presence
P Precipitated
* Single plates were used for this test

TABLE 2

- Mutation test 1
Mean revertant colonies obtained

Material	Test concentration (µg active ingredient /plate)	With or without S-9	Reverse mutation (number of colonies/plate) *				
			Base pair exchange type			Frame shift type	
			TA 100	TA 1535	WP2 uvrA	TA 98	TA 1537
Solvent control		-	151	17	69	27	11
	5000.0	-	220P	11P	71P	23P	5P
	2500.0	-	167P	20P	64P	26P	10P
	1250.0	-	147P	13P	63P	27P	9P
	625.0	-	145P	16P	72P	25P	7P
	312.5	-	137P	19P	73P	28P	9P
	0.0	-	158	19	72	27	10
Solvent control		+	136	20	69	29	12
	5000.0	+	1165P	143P	73P	29P	8P
	2500.0	+	441P	75P	53P	22P	11P
	1250.0	+	285P	50P	73P	27P	7P
	625.0	+	226P	16P	73P	30P	9P
	312.5	+	154P	18P	65P	28P	9P
	0.0	+	155	20	69	29	11
Positive controls	Name	Not Requiring S-9 mix	ENNG	ENNG	ENNG	NF	9 AC
	Concentration (µg/plate)		3.0	5.0	2.0	1.0	80.0
	Number of colonies/plate	-	548	288	770	349	X
	Name	Requiring S-9 mix	AA	AA	AA	AA	AA
	Concentration (µg/plate)		1.0	2.0	10.0	0.5	2.0
	Number of colonies/plate	+	527	185	340	126	80

−	Absence
+	Presence
P	Precipitated
X	Too many colonies to count accurately
ENNG	*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine
9 AC	9-Aminoacridine
NF	2-Nitrofluorene
AA	2-Aminoanthracene
*	Values are the mean of 3 plates, for individual plate data see Tables 3 and 4

TABLE 3

Mutation test 1

- revertant colony counts obtained per plate using bacterial strains TA 100, TA 1535, WP2 *uvr*A, TA 98 and TA 1537

Strain	Dose level (µg active ingredient /plate)	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	Solvent	-	151	15.9	133, 162, 159
	5000.0	-	220	22.7	224P, 196P, 241P
	2500.0	-	167	14.2	162P, 183P, 156P
	1250.0	-	147	24.2	130P, 137P, 175P
	625.0	-	145	20.8	169P, 134P, 132P
	312.5	-	137	20.7	159P, 133P, 118P
	0.0	-	158	15.1	175, 151, 147
	Solvent	+	136	10.0	146, 135, 126
	5000.0	+	1165	183.5	1303P, 957P, 1236P
	2500.0	+	441	114.3	571P, 398P, 355P
	1250.0	+	285	11.2	297P, 275P, 282P
	625.0	+	226	14.2	239P, 211P, 229P
	312.5	+	154	8.0	162P, 154P, 146P
	0.0	+	155	12.9	170, 150, 146
TA 1535	Solvent	-	17	2.3	18, 14, 18
	5000.0	-	11	1.5	12P, 11P, 9P
	2500.0	-	20	1.2	19P, 21P, 19P
	1250.0	-	13	4.7	15P, 17P, 8P
	625.0	-	16	6.0	10P, 15P, 22P
	312.5	-	19	2.3	18P, 22P, 18P
	0.0	-	19	4.0	21, 21, 14
	Solvent	+	20	0.0	20, 20, 20
	5000.0	+	143	38.3	185P, 110P, 134P
	2500.0	+	75	4.5	70P, 79P, 75P
	1250.0	+	50	8.7	54P, 56P, 40P
	625.0	+	16	4.2	13P, 15P, 21P
	312.5	+	18	2.6	20P, 15P, 19P
	0.0	+	20	2.1	18, 22, 19
WP2 uvrA	Solvent	-	69	6.1	62, 73, 72
	5000.0	-	71	21.0	92P, 50P, 71P
	2500.0	-	64	12.5	65P, 76P, 51P
	1250.0	-	63	12.0	75P, 51P, 62P
	625.0	-	72	7.6	81P, 67P, 69P
	312.5	-	73	12.2	86P, 62P, 70P
	0.0	-	72	11.9	80, 58, 77
	Solvent	+	69	16.0	52, 70, 84
	5000.0	+	73	9.1	83P, 72P, 65P
	2500.0	+	53	7.0	50P, 61P, 48P
	1250.0	+	73	17.7	89P, 76P, 54P
	625.0	+	73	13.6	62P, 68P, 88P
	312.5	+	65	3.5	65P, 62P, 69P
	0.0	+	69	7.8	63, 67, 78

− Absence
\+ Presence
SD Standard deviation
P Precipitated

TABLE 3

(continued)

Mutation test 1

- revertant colony counts obtained per plate using bacterial strains TA 100, TA 1535, WP2 *uvr*A, TA 98 and TA 1537

Strain	Dose level (µg active ingredient /plate)	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 98	Solvent	-	27	3.6	31, 24, 26
	5000.0	-	23	4.5	23P, 18P, 27P
	2500.0	-	26	4.6	31P, 23P, 23P
	1250.0	-	27	0.6	27P, 27P, 28P
	625.0	-	25	6.1	20P, 24P, 32P
	312.5	-	28	1.5	29P, 28P, 26P
	0.0	-	27	1.5	25, 28, 27
	Solvent	+	29	5.3	23, 31, 33
	5000.0	+	29	7.9	20P, 35P, 32P
	2500.0	+	22	5.9	20P, 18P, 29P
	1250.0	+	27	5.1	23P, 33P, 26P
	625.0	+	30	1.5	30P, 29P, 32P
	312.5	+	28	1.5	30P, 28P, 27P
	0.0	+	29	2.5	29, 31, 26
TA 1537	Solvent	-	11	2.3	12, 8, 12
	5000.0	-	5	3.5	9P, 2P, 5P
	2500.0	-	10	2.1	8P, 11P, 12P
	1250.0	-	9	2.6	11P, 6P, 10P
	625.0	-	7	3.2	6P, 5P, 11P
	312.5	-	9	5.5	6P, 15P, 5P
	0.0	-	10	0.6	10, 11, 10
	Solvent	+	12	1.5	12, 13, 10
	5000.0	+	8	4.7	4P, 6P, 13P
	2500.0	+	11	1.5	11P, 12P, 9P
	1250.0	+	7	1.5	8P, 5P, 7P
	625.0	+	9	3.2	5P, 11P, 10P
	312.5	+	9	0.6	10P, 9P, 9P
	0.0	+	11	1.5	10, 13, 11

− Absence
+ Presence
SD Standard deviation
P Precipitated

TABLE 4

Mutation test 1

Revertant colony counts obtained per plate with positive control compounds

Strain	Compound	Dose level (µg/plate)	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	ENNG	3.0	-	548	41.5	593, 541, 511
TA 1535	ENNG	5.0	-	288	8.5	280, 297, 288
WP2 uvrA	ENNG	2.0	-	770	47.8	823, 757, 730
TA 98	NF	1.0	-	349	26.0	364, 364, 319
TA 1537	9 AC	80.0	-	-	-	X, X, X
TA 100	AA	1.0	+	527	29.1	494, 548, 540
TA 1535	AA	2.0	+	185	68.5	264, 139, 153
WP2 uvrA	AA	10.0	+	340	55.6	283, 394, 343
TA 98	AA	0.5	+	126	33.5	100, 115, 164
TA 1537	AA	2.0	+	80	5.1	81, 74, 84

−	Absence
+	Presence
SD	Standard deviation
X	Too many colonies to count accurately
ENNG	*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine
9 AC	9-Aminoacridine
NF	2-Nitrofluorene
AA	2-Aminoanthracene

TABLE 5

- Mutation test 2
Mean revertant colonies obtained

Material	Test concentration (µg active ingredient /plate)	With or without S-9	Reverse mutation (number of colonies/plate) *				
			Base pair exchange type			Frame shift type	
			TA 100	TA 1535	WP2 uvrA	TA 98	TA 1537
Solvent control		-	148	16	75	25	11
	5000.0	-	225P	18P	75P	26P	12P
	2500.0	-	113P	26P	57P	23P	9P
	1250.0	-	138P	18P	65P	28P	10P
	625.0	-	110P	17P	68P	24P	12P
	312.5	-	137P	18P	66P	28P	9P
	0.0	-	143	17	74	31	9
Solvent control		+	153	16	66	25	9
	5000.0	+	273P	126P	62P	130P	9P
	2500.0	+	241P	65P	64P	78P	8P
	1250.0	+	216P	40P	58P	44P	9P
	625.0	+	210P	22P	57P	51P	8P
	312.5	+	172P	19P	55P	45P	14P
	0.0	+	144	17	82	28	12
Positive controls	Name	Not Requiring S-9 mix	ENNG	ENNG	ENNG	NF	9 AC
	Concentration (µg/plate)		3.0	5.0	2.0	1.0	80.0
	Number of colonies/plate	-	405	185	721	288	X
	Name	Requiring S-9 mix	AA	AA	AA	AA	AA
	Concentration (µg/plate)		1.0	2.0	10.0	0.5	2.0
	Number of colonies/plate	+	429	132	280	88	79

−	Absence
+	Presence
P	Precipitated
X	Too many colonies to count accurately
ENNG	*N*-Ethyl-*N*'-nitro-*N*-nitrosoguanidine
9 AC	9-Aminoacridine
NF	2-Nitrofluorene
AA	2-Aminoanthracene
*	Values are the mean of 3 plates, for individual plate data see Tables 6 and 7

TABLE 6

Mutation test 2

- revertant colony counts obtained per plate using bacterial strains TA 100, TA 1535, WP2 *uvr*A, TA 98 and TA 1537

Strain	Dose level (µg active ingredient /plate)	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	Solvent	-	148	8.7	144, 158, 142
	5000.0	-	225	11.2	221P, 238P, 217P
	2500.0	-	113	16.8	132P, 100P, 107P
	1250.0	-	138	20.8	114P, 146P, 153P
	625.0	-	110	12.2	121P, 97P, 113P
	312.5	-	137	6.8	145P, 132P, 135P
	0.0	-	143	11.4	156, 140, 134
	Solvent	+	153	5.3	155, 147, 157
	5000.0	+	273	20.6	253P, 294P, 271P
	2500.0	+	241	16.9	229P, 233P, 260P
	1250.0	+	216	11.5	229P, 212P, 207P
	625.0	+	210	30.0	175P, 228P, 226P
	312.5	+	172	19.7	194P, 156P, 166P
	0.0	+	144	17.7	164, 132, 135
TA 1535	Solvent	-	16	3.5	13, 16, 20
	5000.0	-	18	2.6	17P, 16P, 21P
	2500.0	-	26	3.1	27P, 29P, 23P
	1250.0	-	18	1.5	18P, 17P, 20P
	625.0	-	17	4.7	15P, 13P, 22P
	312.5	-	18	7.6	20P, 10P, 25P
	0.0	-	17	2.6	18, 19, 14
	Solvent	+	16	4.0	14, 14, 21
	5000.0	+	126	42.6	147P, 77P, 154P
	2500.0	+	65	12.1	51P, 72P, 72P
	1250.0	+	40	4.5	44P, 40P, 35P
	625.0	+	22	6.7	15P, 28P, 24P
	312.5	+	19	3.1	16P, 22P, 18P
	0.0	+	17	2.1	15, 19, 18
WP2 uvrA	Solvent	-	75	11.4	84, 62, 78
	5000.0	-	75	10.3	72P, 66P, 86P
	2500.0	-	57	14.2	47P, 73P, 50P
	1250.0	-	65	14.6	67P, 78P, 49P
	625.0	-	68	17.6	48P, 78P, 79P
	312.5	-	66	5.5	66P, 71P, 60P
	0.0	-	74	5.9	70, 81, 72
	Solvent	+	66	4.0	68, 61, 68
	5000.0	+	62	4.2	57P, 63P, 65P
	2500.0	+	64	6.7	57P, 70P, 66P
	1250.0	+	58	1.2	59P, 57P, 57P
	625.0	+	57	5.2	60P, 60P, 51P
	312.5	+	55	7.1	56P, 61P, 47P
	0.0	+	82	7.8	88, 84, 73

− Absence
\+ Presence
SD Standard deviation
P Precipitated

TABLE 6

(continued)

Mutation test 2

- revertant colony counts obtained per plate using bacterial strains TA 100, TA 1535, WP2 *uvr*A, TA 98 and TA 1537

Strain	Dose level (µg active ingredient /plate)	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 98	Solvent	-	25	1.2	26, 24, 24
	5000.0	-	26	6.1	27P, 19P, 31P
	2500.0	-	23	5.7	21P, 18P, 29P
	1250.0	-	28	2.5	26P, 28P, 31P
	625.0	-	24	3.6	27P, 20P, 25P
	312.5	-	28	3.0	28P, 31P, 25P
	0.0	-	31	2.1	33, 30, 29
	Solvent	+	25	3.2	23, 24, 29
	5000.0	+	130	35.6	120P, 101P, 170P
	2500.0	+	78	3.2	77P, 76P, 82P
	1250.0	+	44	7.5	48P, 48P, 35P
	625.0	+	51	5.6	52P, 56P, 45P
	312.5	+	45	12.6	58P, 43P, 33P
	0.0	+	28	3.6	31, 24, 29
TA 1537	Solvent	-	11	3.2	7, 12, 13
	5000.0	-	12	1.5	11P, 12P, 14P
	2500.0	-	9	3.2	10P, 11P, 5P
	1250.0	-	10	2.5	13P, 10P, 8P
	625.0	-	12	4.2	7P, 13P, 15P
	312.5	-	9	3.2	11P, 5P, 10P
	0.0	-	9	2.5	7, 12, 9
	Solvent	+	9	2.3	12, 8, 8
	5000.0	+	9	5.8	6P, 16P, 6P
	2500.0	+	8	1.2	9P, 7P, 9P
	1250.0	+	9	1.5	11P, 9P, 8P
	625.0	+	8	2.0	10P, 6P, 8P
	312.5	+	14	2.1	13P, 12P, 16P
	0.0	+	12	2.6	14, 13, 9

– Absence
\+ Presence
SD Standard deviation
P Precipitated

TABLE 7

Mutation test 2

Revertant colony counts obtained per plate with positive control compounds

Strain	Compound	Dose level (µg/plate)	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	ENNG	3.0	-	405	32.3	381, 393, 442
TA 1535	ENNG	5.0	-	185	7.4	191, 177, 188
WP2 uvrA	ENNG	2.0	-	721	29.4	688, 729, 745
TA 98	NF	1.0	-	288	42.0	319, 240, 304
TA 1537	9 AC	80.0	-	-	-	X, X, X
TA 100	AA	1.0	+	429	8.2	436, 431, 420
TA 1535	AA	2.0	+	132	14.7	145, 135, 116
WP2 uvrA	AA	10.0	+	280	15.3	293, 263, 283
TA 98	AA	0.5	+	88	6.0	82, 87, 94
TA 1537	AA	2.0	+	79	14.2	74, 95, 68

–	Absence
+	Presence
SD	Standard deviation
X	Too many colonies to count accurately
ENNG	*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine
9 AC	9-Aminoacridine
NF	2-Nitrofluorene
AA	2-Aminoanthracene

APPENDIX 1

Summary report

1. GENERAL INFORMATION (information available to testing facility)

Name of chemical (IUPAC nomenclature)					
Alternative names		Physico-chemical properties of the new chemical substance	Molecular weight		
			Appearance at ordinary temperature		powder
			Stability		
Structural formula or empirical formula (or outline of manufacturing method in case both are unknown)			Melting point		
			Boiling point		
			Vapour pressure		
CAS number	No		Partition coefficient		
Lot number	G1113A1870		Solubility	50 mg active ingredient/ml	
Purity	89.0%		Degree of solubility	Water	No
Name and concentration of impurities				DMSO	Insoluble
				Acetone	Insoluble
				Other Ethanol DMF *	Insoluble Soluble

DMF * Dimethyl formamide

2. TESTER STRAINS

(1) Source

Bacterial strain	Obtained from	Date	Date frozen batch checked for strain characteristics
TA 1535	Prof. B.N. Ames, University of California, Berkeley, California, U.S.A..	30 August 1979	29 March 1996
TA 1537		30 August 1979	29 March 1996
TA 98		30 August 1979	29 March 1996
TA 100		9 November 1979	29 March 1996
WP2 *uvr*A	National Collection of Industrial Bacteria, Aberdeen, Scotland.	17 August 1978	29 March 1996

APPENDIX 1

(continued)

(2) Storage

Storage	2. Large scale freezing	
Storage temperature	*c.* −80°C	
Composition	Bacterial suspension: 0.2 ml	DMSO: 0.0175 ml
	Other ( ): None	

3. S-9 MIX

(1) Source of S-9

1. Made in-house	Prepared on	14 May 1996

(2) Storage of S-9

Storage temperature	*c.* −80°C	Name and model of storage apparatus	ScienTemp −85°C 88 L chest freezer, model 85-3.1

(3) Preparation of S-9

Animal used		Inducing substance	
Species Strain	Rat Sprague-Dawley	Name	Aroclor 1254
Sex	Male	Administration method	Single intraperitoneal injection
Age (in weeks)	7 - 8	Administration period and amount	5 days
Weight	<300 g		500 mg/kg body-weight

(4) Composition of S-9 mix

Constituents	Amount in 1 ml S-9 mix	Constituents	Amount in 1 ml S-9 mix
S-9	0.1 ml	NADPH	4 μmol
$MgCl_2$	8 μmol	NADH	4 μmol
KCl	33 μmol	Na-phosphate buffer	100 μmol
Glucose-6-phosphate	5 μmol		

APPENDIX 1

(continued)

4. POSITIVE CONTROL SUBSTANCES AND THEIR SOLVENTS

Substance		Supplier	Lot number	Grade	Purity	Solvent
Positive control substance	*N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine	Sigma	67F-3700	Standard laboratory reagent	98%	DMSO
	9-Aminoacridine	Sigma	96F-05641		>98%	DMSO
	2-Nitrofluorene	Aldrich	61896		98%	DMSO
	2-Aminoanthracene	Aldrich	0013406		96%	DMSO
Solvent	Dimethyl sulphoxide	Fisons FSA	9506286325	Analytical reagent	99.8%	
Preparation and storage of positive control solutions		2. Stock solutions prepared periodically and stored at 4°C				
Positive control stock solutions prepared		First main test: 17 June 1996 Second main test: 17 June 1996				

5. PREPARATION OF THE TEST SUBSTANCE SOLUTION

Solvent used	Name	Manufacturer	Lot number	Grade	Purity
	DMF	Fisons FSA	43	AR	99%
Stability of the test substance in the solvent	Not assessed in this test				
Reason for selection of solvent	Soluble				
Method of suspension *etc.* when test substance is difficult to dissolve					
Storage time and temperature from preparation of the solution to dosing	Dosed within 1 hour of preparation and stored at room temperature during this time				
Purity conversion (indicate appropriate response)	Yes		No ✓		

APPENDIX 1

(continued)

6. CONDITIONS OF PRE-CULTURE

(1) Conditions

Nutrient broth		: Name	Manufacturer	Lot number
		DAB 7	Merck Ltd.	V430234406
Pre-culture time and temperature		10 hours at 37°C		
Storage time and temperature from inoculation of stock strains to shaking of culture		*c.* 6 hours at room temperature		
Storage time and temperature from completion of shaking to usage		*c.* 4 hours at 4°C		
Model and manufacturer of culture shaking apparatus		Luckham R300 Benchtop Incubator Shaker		
Shaking method (style and frequency)		Circular shaking at *c.* 200 revs per minute		
Culture vessel (shape and capacity)		100 ml Medical flat bottles		
Volume of culture liquid		25 ml		
Inoculum volume	200 µl	Inoculated cell number	*c.* 4 x 10^6 cells	

(2) Number of cells at completion of pre-culture

		Base pair exchange type			Frame shift type	
		TA 100	TA 1535	WP2 *uvr*A	TA 98	TA 1537
Bacterial cell count (x 10^9/ml)	Dose range finding study	4.2	3.9	11.5	4.7	5.0
	First main test	3.9	4.4	11.3	4.2	4.7
	Second main test	3.8	5.0	11.4	4.7	4.2
Measuring method		1. Conversion from O.D. value				

7. AGAR PLATE MEDIUM

(1) Top agar

Agar	Name	Agar (bacteriological grade)
	Manufacturer	Gibco Europe Ltd., Paisley, Scotland.
	Lot number	20H2163B

APPENDIX 1

(continued)

(2) Minimal glucose agar

1. Made in-house	Prepared on 3 June 1996 Toxicity test 13 & 14 June 1996 First test 21 & 24 June 1996 Second test
Name of agar used, manufacturer, lot number, *etc.*	Bacteriological grade, Gibco Europe Ltd., Lot number: V430234406
Volume of agar plate medium	25 ml

8. STERILITY TEST (indicate appropriate response)

	Bacterial contamination	
Test substance solution	Yes	No ✓
S-9 Mix	Yes	No ✓

9. TEST METHOD

(1) Test method (indicate method used)

1. Pre-incubation method
2. Plate method ✓

(2) Test condition

		Pre-incubation method	Plate method
Composition	Bacterial suspension	N/A	0.1 ml
	Test substance solution	N/A	0.1 ml
	Na-phosphate buffer	N/A	0.5 ml
	S-9 mix (in case of metabolic activation method)	N/A	0.5 ml
	Top agar solution	N/A	2.0 ml
	Others ( )	N/A	None
Pre-incubation	Temperature	N/A	N/A
	Time	N/A	N/A
Incubation	Temperature	N/A	37°C
	Time	N/A	*c.* 72 hours

APPENDIX 1

(continued)

10. METHOD OF COLONY COUNTING (indicate method used)

	1. Manual measurement 2. Mechanical measurement ✓
Reason for the combined use of methods 1 and 2 if applicable	
Model and supplier of colony counter	Seescan model COL Colony counter
Correction method	1. No correction

11. TEST RESULTS

(1) Test results are reported in Tables 1 - 7
(2) Judgement of the results

Judgement (indicate one)	Positive ✓	Negative
Reason for judgement: Substantial dose-related increases in revertant colony numbers were observed with TA 1535, TA 98 and TA 100 in the presence of S-9 mix.		

(3) Comments

It is concluded that, when tested in dimethyl formamide, in this bacterial system.	shows evidence of mutagenic activity

APPENDIX 1

(continued)

12. OTHERS

Testing institution	Name	Huntingdon Life Sciences Ltd		
	Address	Huntingdon, Cambridgeshire, Gt. Britain. Tel.: 01480-890431 Fax.: 01480-890693		
Administrator	Name/Title	Dr. J.A. Allen Director, Laboratory Sciences Division		
Quality Assurance Director (Also responsible for archives)	Name/Title	Dr. D.J. Ford Quality Director		
Study Director	Name/Title	Miss Ricarda A. Gant Study Director Department of Cellular Toxicology		
	Years of experience	8 years	Final educational career and specialised field	University of Birmingham Biological Sciences
Study Supervisor	Miss Ricarda A. Gant (details as above)			
Test dates	From 4 to 28 June 1996			
Test number	TDK 31B			